THE INFLUENCE OF CLAY MINERALS ON THE ENZYMATIC HYDROLYSIS OF ORGANIC PHOSPHORUS COMPOUNDS

BY

MAX MERLE MORTLAND

B.S., University of Illinois, 1946
M.S., University of Illinois, 1947
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AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
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THE INFLUENCE OF CLAY MINERALS ON THE ENZYMATIC HYDROLYSIS OF ORGANIC PHOSPHORUS COMPOUNDS

Organic phosphorus compounds contain a large portion of the total phosphorus in many soils. Mineralization, or the release of inorganic phosphate from organic phosphorus compounds, has been demonstrated by several workers. The inorganic phosphate produced by mineralization is available for plant use. Since an enzymatic influence has been ascribed to this reaction, the factors affecting it are of importance.

Ensminger and Gieseking, and Erickson have studied enzymatic hydrolysis of proteins, amino acids, and simpler molecules in the presence of clay minerals. It was the purpose of this investigation to extend these studies to organic phosphorus compounds and enzymes capable of hydrolizing them.

The effect of various clays on the enzymatic hydrolysis of organic phosphorus compounds was studied. Fructosediphosphatase, phytase, glycerophosphatase, and lecithinase were the enzymes used for the hydrolytic reactions. These enzymes were prepared from bread yeast, wheat bran, bread yeast, and kidney cortex respectively. The various clays studied included kaolinite, Wyoming Bentonite (montmorillonite), Swygert (illite), and Cisne (a clay similar to Putnam). The activity of the enzyme was determined with no clay present, and then in the presence of various amounts and kinds of clays. The enzymatic reactions were performed paying special attention to the control of pH, temperatures, inhibitors, activators, and concentrations. The difference between the activity of the enzyme on the organic phosphorus compound in the presence of a suitable buffer and the activity in the presence of clays was considered a measure of the influence of the clay upon the enzyme activity. All of the clays studied exhibited an inhibiting influence upon the enzymatic hydrolysis of organic phosphorus compounds.

A study was made of the nature of the inhibition by clays of the enzymatic hydrolysis of organic phosphorus compounds. A digestion cell was prepared containing two chambers separated by a membrane permeable to glycerophosphate and inorganic phosphate, but not to clays or enzymes. The enzyme was placed in one chamber, and glycerophosphate and Wyoming Bentonite in the other. In order for hydrolysis to occur, the glycerophosphate had to diffuse through the membrane into the enzyme chamber. The inorganic phosphate was determined in both chambers over a period of time. Hydrolysis of the glycerophosphate occurred and inorganic phosphate appeared in both chambers of the cell. The Wyoming Bentonite concentration which was used, totally inhibited hydrolysis when placed in the same cell with the enzyme and glycerophosphate.

The following conclusions were drawn from this investigation:

Clays inhibit enzymatic hydrolysis of organic phosphorus compounds. The amount of inhibition varies with the kind of clay according to the following series, Wyoming Bentonite (montmorillonite) > Cisne(Putnam - like clay) > Swygert (illite) > kaolinite.

The membrane study indicates that the inhibition is due to the effect of the clay on the enzyme and not to adsorption of the organic phosphorus compound by the clay. This study also indicates that phosphatases within dead or living plant, animal, or microbiological cells may hydrolyze organic phosphorus compounds which can diffuse through their cell walls. The resulting inorganic phosphate may diffuse back into the soil solution, or be used for metabolic purposes in the living organism.

The inhibition of phosphatase activity by clays is roughly proportional to the base exchange capacity of the clay. A constant, evaluated for each clay in an empirical equation which describes hydrolysis of the organic compound in the presence of varying quantities of clay, is approximately equal

to the ratios of the base exchange capacities of the various clays.

In general this study indicates that extracellular phosphatases in soils, may be prevented from hydrolyzing organic phosphorus compounds because of inhibition by clays. The mineralization of organic phosphorus compounds in soils reported by other workers, may be the result of diffusion of the compounds into cells containing phosphatases, and subsequent diffusion of the inorganic phosphate back through the cell wall into the soil solution.

VITA

The author was born March 30, 1923, in Streator, Illinois. He attended rural grammar school, and Streator Township High School, where he graduated in June, 1940. In the fall of 1940, he entered the University of Illinois. During July, 1943, he entered Purdue University in the Navy V - 12 program, and was commisioned an officer after attending the Columbia University Midshipman's School.

After completing his service in the Navy, he reentered the University of Illinois. He received his Bachelor of Science degree in Agriculture in September, 1946, Master of Science degree in Agronomy in June, 1947, and Master of Science degree in Chemistry in August, 1950.

Since the summer of 1947 he has been Assistant and First Assistant in the Soil Survey at the University of Illinois.

He is a member of Gamma Sigma Delta and Sigma Xi.

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